

Appl. No. 09/863,693
Amendment dated December 17, 2004
Reply to Advisory Action of October 28, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1-51. canceled

52. (new) A method of preparing a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) each variable light chain polypeptide is selected to have a common sequence;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) each variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for a first antigen, each variable light chain polypeptide interacts with a second antibody heavy chain polypeptide to form an antigen binding domain for the second antigen, wherein the first and second antigens are different from one another, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody; the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide, the second polypeptide, and the variable light chain polypeptide, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

53. (new) The method of claim 52, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

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54. (new) The method of claim 53, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

55. (new) The method of claim 52, wherein the first and second multimerization domains each comprise an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance-into-cavity interaction, or a free thiol which forms an intermolecular disulfide bond;

56. (new) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

- (a) each variable light chain polypeptide is selected to have a common sequence;
- (b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;
- (c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and
- (d) each variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for a first antigen, the variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for a second antigen, the first and second antigens are different from one another, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody.

57. (new) The host cell of claim 57, wherein the cell is a mammalian cell.

58. (new) A method of preparing a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

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(a) each variable light chain polypeptide has three CDRs and is selected to have at least 98% sequence identity with the other variable light chain of the bispecific antibody, wherein the variable light chain polypeptides differ from one another at amino acid positions outside of the CDRs;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) each variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for a first antigen, each variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for a second antigen, the first and second antigens are different from one another, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody; the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide, the second polypeptide, and the variable light chain polypeptide, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

59. (new) The method of claim 58, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

60. (new) The method of claim 59, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

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61. (new) The method of claim 58, wherein each of the antibody variable light chain polypeptides have the same sequence.

62. (new) The method of claim 58, wherein the antibody variable light chain polypeptide has at least 99% sequence identity to the other antibody variable light chain polypeptide.

63. (new) The method of claim 58, wherein the first and second multimerization domain each comprise an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance-into-cavity interaction, or a free thiol which forms an intermolecular disulfide bond.

64. (new) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) each variable light chain polypeptide has three CDRs and is selected to have at least 98% sequence identity with other variable light chain of the bispecific antibody, wherein the variable light chain polypeptides differ at amino acid positions outside of the CDRs;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) each variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for a first antigen, each variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for a second antigen, the first and second antigens are different from one another, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody.

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65. (new) The host cell of claim 64, wherein the cell is a mammalian cell.

66. (new) A method of preparing a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) the variable light chain polypeptide is selected to have a common sequence from a first antibody variable light chain specific for a first antigen and from a second antibody variable light chain specific for a second antigen, and the first and second antigens differ from one another;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) the variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for the first antigen, the variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for the second antigen, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody; the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide, the second polypeptide, and the variable light chain polypeptide, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

67. (new) The method of claim 66, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

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68. (new) The method of claim 67, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

69. (new) The method of claim 66, wherein the antibody variable light chain polypeptide is identical to the first antibody variable light chain and to the second antibody variable light chain.

70. (new) The method of claim 66, wherein the antibody variable light chain has at least 98% sequence identity to the first antibody variable light chain and the second antibody variable light chain and only differs from the first and second antibody variable light chains at amino acid positions outside of three CDRs.

71. (new) The method of claim 66, wherein the first and second multimerization domain each comprise an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance-into-cavity interaction, or a free thiol which forms an intermolecular disulfide bond;

72. (new) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) the variable light chain polypeptide is selected to have a common sequence from a first antibody variable light chain specific for a first antigen and from a second antibody variable light chain specific for a second antigen, and the first and second antigens differ from one another;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

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(d) the variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for the first antigen, the variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for the second antigen, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody.

73. (new) The host cell of claim 72, wherein the cell is a mammalian cell.

74. (new) A method of preparing a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) the variable light chain polypeptide has three CDRs and is selected to have at least 98% sequence identity to a first antibody variable light chain specific for a first antigen and to a second antibody variable light chain specific for a second antigen, wherein the variable light chain polypeptide differs from the first and second antibody variable light chain polypeptide at amino acid positions outside of the CDRs, and the first and second antigens are different from one another;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) the variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for the first antigen, the variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for the second antigen, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody; the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide, the second polypeptide, and the variable light chain

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polypeptide, wherein the culturing is such that the nucleic acid is expressed ;and

(ii) recovering the bispecific antibody from the host cell culture.

75. (new) The method of claim 74, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

76. (new) The method of claim 75, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

77. (new) The method of claim 74, wherein the antibody variable light chain polypeptide is identical to a first antibody variable light chain and a second antibody variable light chain.

78. (new) The method of claim 74, wherein the antibody variable light chain has at least 99% sequence identity to the first antibody variable light chain and to the second antibody variable light chain.

79. (new) The method of claim 74, wherein the first and second multimerization domain each comprise an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance-into-cavity interaction, or a free thiol which forms an intermolecular disulfide bond.

80. (new) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide, a second polypeptide and a variable light chain polypeptide, wherein

(a) the variable light chain polypeptide has three CDRs and is selected to have at least 98% sequence identity to a first antibody variable light chain

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specific for a first antigen and to a second antibody variable light chain specific for a second antigen, wherein the variable light chain polypeptide differs from the first and second antibody variable light chain polypeptides at amino acid positions outside of the CDRs, and the first and second antigens are different from one another;

(b) the first polypeptide comprises a first antibody variable heavy chain polypeptide and a first multimerization domain;

(c) the second polypeptide comprises a second antibody variable heavy chain polypeptide and a second multimerization domain; and

(d) the variable light chain polypeptide interacts with the first antibody heavy chain polypeptide to form an antigen binding domain for the first antigen, the variable light chain polypeptide interacts with the second antibody heavy chain polypeptide to form an antigen binding domain for the second antigen, and the first and second multimerization domains of the first and second polypeptides interact to form a bispecific antibody.

81. (new) The host cell of claim 80, wherein the cell is a mammalian cell.

82. (new) A method of preparing a bispecific antibody comprising:

(a) a variable light chain obtained by screening a library of antibody variable domains, and selecting the variable light chain to have at least 98% sequence identity to each variable light chain domain of a first and second antibody, wherein the first and second antibody bind to different antigens;

(b) culturing a host cell comprising one or more nucleic acids encoding: a first polypeptide comprising a first variable heavy chain polypeptide from the first antibody and a first multimerization domain; a second polypeptide comprising a second variable heavy chain polypeptide from the second antibody and a second multimerization domain, and the selected variable light chain; wherein the nucleic acid is expressed to form the first polypeptide, the second polypeptide, and the variable light chain, wherein the first variable heavy

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chain variable domain and the variable light chain domain form a first binding site for a first antigen and the second variable heavy chain domain and the variable light chain form a second binding site for a second antigen; and the first and second multimerization domains interact to form a bispecific antibody; and

(c) recovering the bispecific antibody from the cell culture.

83. (new) The method of claim 82, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

84. (new) The method of claim 83, wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

85. (new) The method of claim 82, wherein the variable light chain is identical to each variable light chain domain of a first and second antibody.

86. (new) The method of claim 82, wherein the antibody variable light chain has at least 98% sequence identity to each variable light chain domain of a first and second antibody.

87. (new) The method of claim 82, wherein the first and second multimerization domain each comprise an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance-into-cavity interaction, or a free thiol which forms an intermolecular disulfide bond.